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Application of microbial electrolysis cells to treat spent yeast from an alcoholic fermentation

Ornella Sosa-Hernández [a], Sudeep C. Popat [b], Prathap Parameswaran [b], Gibrán Sidney Alemán-Nava [a], César I. Torres [c], Germán Buitrón [d], Roberto Parra-Saldívar [a,*]

[a] Tecnologico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico
[b] Biodesign Institute, Swette Center for Environmental Biotechnology, Arizona State University, 1001 S McAllister Ave, Tempe, AZ 85287, USA
[c] School for Engineering of Matter, Transport and Energy, Arizona State University, 1001 S McAllister Ave, Tempe, AZ 85287, USA
[d] Unidad Académica Juriquilla, Instituto de Ingeniería, Universidad Nacional Autónoma de México, Blvd. Juriquilla 3001, Querétaro, Qro 76230, Mexico

HIGHLIGHTS

- Electron recovery from spent yeast was improved by ethanol addition in a MEC.
- Current density and hydrogen production increased with higher organic loads.
- Spent yeast treatment was mostly fermentative explaining the drift of electrons.
- Ethanol and experimental conditions could have induced spent yeast autolysis.
- This is the first study, as far as we know, of spent yeast treated in a MEC.

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ABSTRACT

Spent yeast (SY), a major challenge for the brewing industry, was treated using a microbial electrolysis cell to recover energy. Concentrations of SY from bench alcoholic fermentation and ethanol were tested, ranging from 750 to 1500 mg COD/L and 0 to 2400 mg COD/L respectively. COD removal efficiency (RE), coulombic efficiency (CE), coulombic recovery (CR), hydrogen production and current density were evaluated. The best treatment condition was 750 mg COD/L SY + 1200 mg COD/L ethanol giving higher COD RE, CE, CR (90 ± 1%, 90 ± 2% and 81 ± 1% respectively), as compared with 1500 mg COD/L SY (76 ± 2%, 63 ± 7% and 48 ± 4% respectively); ethanol addition was significantly favorable (p value = 0.011), possibly due to electron availability and SY autolysis. 1500 mg COD/L SY + 1200 mg COD/L ethanol achieved higher current density (222.0 ± 31.3 A/m^3) and hydrogen production (2.18 ± 0.66 L_{H_2}/day/$L_{Reactor}$) but with lower efficiencies (87 ± 2% COD RE, 71.0±.4% CE). Future work should focus on electron sinks, acclimation and optimizing SY breakdown.

1. Introduction

The brewing industry generates significant volumes of by-products and solid waste, the disposal and management of which represent important operational costs and environmental challenges. Approximately 1.5–3% of the total volume of beer produced corresponds to spent yeast (SY) (Fillaudeau et al., 2006) which is considered the second major by-product from breweries (Huige, 2006). Before disposal, brewer's SY requires an inactivation treatment, which can be energy intensive as it requires the addition of toxic substances and heating at high temperatures. It is difficult to treat SY as a liquid waste since it increases the biological oxygen demand (BOD) of any body of water where it is released, and due to the yeast cell bodies and to the residual ethanol from fermentation, it is considered a pollutant (Doubla et al., 2007).

SY is recovered almost at the end of the brewing process and only a fraction of it can be reused (Olajire, 2012). This by-product includes yeast solids, beer solids, soluble ethanol, and sediment of hops and particles of grains (Rocha et al., 2014) and it has a high content of protein, vitamins and amino acids (Mussato, 2009). After a drying treatment, a small fraction of SY is commercially used for animal feed or nutritional supplement (Fillaudeau et al., 2006; Mussato, 2009), while the fraction that is not used is currently disposed of in landfills or, less frequently, directed to anaerobic digestion treatment plants (Neira and Jeison, 2010).

* Corresponding author.
E-mail address: r.parra@itesm.mx (R. Parra-Saldívar).

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There are only a few publications reporting how SY has been treated to obtain methane through anaerobic digestion; the best results having been obtained when it was co-digested (Bocher et al., 2008; Neira and Jeison, 2010; Zupančič et al., 2012). Using a pretreatment neither improved methane production, nor the speed of SY biodegradation, that requires long retention times (Neira and Jeison, 2010).

When high volumes of SY are produced, alternative uses *in situ* have to be explored, because the costs of transport and storage could become significant. Before inactivation, SY can contain both viable and dead cells (Bokulich et al., 2013), leading to high variability in its composition. In addition, a fraction of the SY cells undergo autolysis after the brewing process, leading to the release of internal cellular contents (Steckley et al., 1979), while the viable yeast cells can still perform their metabolic processes, consuming some of the residual carbohydrates in the media and releasing products like ethanol. These SY characteristics could be advantageous for the degradation process that needs to occur in a microbial electrolysis cell (MEC).

MEC is a developing technology that can allow hydrogen production from organic matter degradation (Logan et al., 2008). In these systems, electrochemically active bacteria can oxidize organic matter and generate CO_2, electrons and protons. These electrons are transferred to an electrode by a specific group of bacteria called anode respiring bacteria (ARB) which can only oxidize specific simple products such as acetate or hydrogen. The electrons travel through a circuit to the cathode where, with a minimum added voltage, hydrogen gas is produced. Fermentation of complex substrates is also possible in an MEC using a chain of microbial anaerobic reactions (Parameswaran et al., 2009) to break those compounds down and generate usable products, obtaining an added value from complex organic wastes (Rittmann, 2008). One of the products thus generated is hydrogen, which is considered a profitable, clean, sustainable and renewable fuel (Kadier et al., 2014). Thus, there is a growing interest in using this technology.

So far, there are no reports about bio-electrochemical systems that include MECs being used to treat brewer's SY. Some authors have reported the use of brewery waste water as a substrate for microbial fuel cells (MFC) (Pant et al., 2010; Wen et al., 2010), finding that the buffering capacity, temperature, and organic load have an important effect on the performance of the process (Feng et al., 2008). Ethanol is one of the main components of the brewery wastes and it has been used as a sole electron donor in MECs to establish a mass and electron balance for the process and to confirm methanogenesis as an important sink of electrons (Parameswaran et al., 2009); this was also confirmed in a pilot-scale MEC using winery waste water, the main component of which was ethanol (Cusick et al., 2011). Since fermentation of complex substrates can occur simultaneously in MEC (Parameswaran et al., 2009), when using mixed cultures (Kadier et al., 2014), an effective acclimation of the inoculum to the new substrate is needed for the best performance of the process. Other factors affecting MEC anode performance fed with complex wastes include organic loading rate and the availability of simple electron donors, such as ethanol or acetate for the ARB rapid utilization (Feng et al., 2008).

The objective of this work was to use MECs to treat SY from a bench scale alcoholic fermentation, and to evaluate the effect of SY and ethanol concentration on MEC performance. The performance of this treatment was evaluated in terms of the organic matter removed (COD) as removal efficiency (COD RE), coulombic efficiency (CE), coulombic recovery (CR), maximum current density (A/m^2 or A/m^3, current normalized to the anode active surface and the current normalized to the anode effective volume respectively) and the rate of hydrogen production (H_2 in $L_{H_2}/day/L_{Reactor}$ or mol/day). This is the first study, as far as we know, that considers the application of MEC for SY treatment.

2. Methodology

2.1. Bench scale alcoholic fermentation

In order to produce a consistent SY sample, a bench scale alcoholic fermentation was performed. Malt extract at 75 g/L (CBW® Pilsen, Briess Malt & Ingredients Co. Chilton, WI, USA) was added to previously boiled water. Once the solution was cold, brewer's yeast (*Saccharomyces cerevisiae*) (0.57 g/L of Safale S-04, Fermentis, France) was added and mixed in a clean container that was closed with an air-lock. The fermentation was carried out at room temperature (23 ± 2 °C) for 2 weeks. At the end of the process, solids were recovered by centrifuging at 3600 rpm for 10 min. The recovered fraction was then diluted in 200 mL of distilled water and stored at 4 °C.

2.2. Chemical characterization of SY

The chemical characterization of the SY was done immediately after the sample was collected and two weeks after that, immediately before the MEC experimental setup, to avoid uncertainty from composition changes during storage. This characterization allowed to monitor the sample stability during storage and to obtain evidence of yeast activity. The characterization included the quantification of total chemical oxygen demand (TCOD), that was measured initially and at the end of each experiment, using HACH procedure and spectrophotometer DR 2010 (HACH, Ames, IA, USA). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to Standard Methods (APHA, 1998). A colorimetric method was used to determine carbohydrate concentration (DuBois et al., 1956) that involved placing 2 mL of sample in a 15 mL culture tube, followed by the addition of 50 μL of 80% phenol solution (w/w) and consecutively 5 mL of 95.5% sulfuric acid. The mixture was incubated for 30 min at room temperature. The specific absorbance at a wavelength of 485 nm was measured with a spectrophotometer, using a previously developed standard calibration curve with glucose. The Lowry method (Lowry et al., 1951) was used to determine proteins concentration. A standard curve with bovine serum albumin was used, with a specific absorbance recorded at 562 nm wavelength. Ammonia ($N\text{-}NH_3$ in mg/L) concentration was determined using the Nessler-HACH method and spectrophotometer DR 2010 (HACH, Ames, IA, USA). The pH was measured using a potentiometer (Orion Star A111, Thermo Scientific, Waltham, MA, USA) and the volatile fatty acids (VFA) and ethanol composition was determined using a gas chromatograph (Model Varian 3300) equipped with a FID detector according to (Buitrón and Carvajal, 2010) and pretreating the samples by filtration (glass filter 0.45 μm, PVDF GD/X, Whatman, GE Healthcare, Ann Arbor, MI, USA) and by acidification with 1 μL of HCl (2 M). The VFAs determined were acetate, butyrate, isobutyrate, iso-valerate and propionate.

2.3. Configuration and MEC operation

H-type MEC reactors (fabricated by Adams & Chittenden Scientific Glass, Berkeley, CA, USA) were used for all the experiments (Fig. 1), each chamber had an effective volume of 310 mL. The anodes were made of brushes of graphite fiber (fabricated by Millrose, Mentor, OH, USA) 9 cm long and 6.5 cm diameter, with approximately 160,000 fibers (Panex 35, Zoltec) and mounted to a titanium wire. To increase the oxidation sites the brushes were

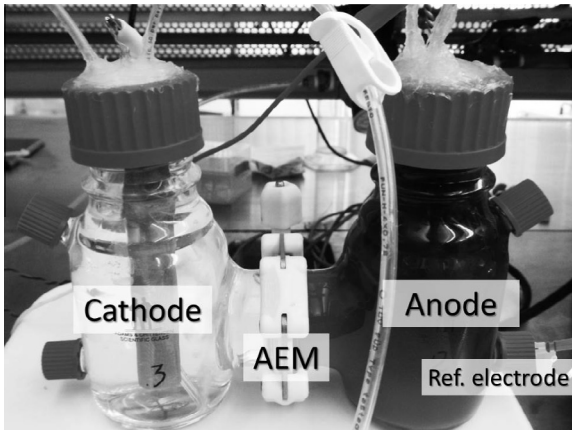

Fig. 1. Components of the MEC reactors used for the experiments. The reactors were sealed with silicon layers to prevent leaking; the anode and cathode chambers were fed through plastic tubes.

treated by soaking them in 0.1 M HNO_3 for 4 h and then in pure acetone overnight, followed by ethanol (95%) for 3 h; then the brushes were washed three times with distilled water before being placed in the MECs. The cathodes consisted of cylinders of 8 cm × 6 cm stainless steel mesh (No. 100) connected to a titanium wire. The two chambers were separated by an anion exchange membrane (AEM) (AEM 7001, Membranes International, Glen Rock, NJ, USA).

Initially the MEC reactors were inoculated with raw pig manure (0.047 g VSS/g TCOD) and mineral media solution (1:3 volume ratio) with acetate (25 mM) as electron donor and phosphate buffer (100 mM) as described by Parameswaran et al. (2009), using NaOH solution (100 mM) as catholyte solution. A reference electrode Ag/AgCl (BASi Electrochemistry, West Lafayette, IN, USA) was placed in the lower part of the anode at about 0.5 cm from the electrode; a potential of −0.3 V vs. Ag/AgCl was maintained at the anode using a potentiostat (ArbOS™ Potentiostat, Arbsource, Phoenix, AZ, USA). The current produced was measured using this equipment and the data was monitored with TracerDaq. Anode and cathode were sparged with N_2 for 2 min before each batch, to remove oxygen. Besides, water used to prepare the solutions was boiled for 10 min to remove dissolved oxygen.

2.4. Acclimation of the microbial community

The acclimation process of the reactors was performed in batch mode, by feeding the previously inoculated reactors with acetate (12.5 mM), ethanol (1200 mg COD/L or 12.5 mM) and SY (1 g/L) consecutively at room temperature (20 ± 4 °C). A magnetic stirrer was used to mix the anode solution. Each substrate batch was repeated until a stable current density was obtained. Once this was observed, the actual experiments were performed.

2.5. Experimental design

Once the microorganisms were acclimated, experiments shown in Table 1 were performed in duplicate. These experiments were designed based on a factorial design 2^2 with the goal of obtaining an insight into the possible advantages of the SY treatment. A constant temperature (31 ± 1 °C) was maintained using a hot air dispenser and a temperature controller, while the mixing of the anode solution was done by recirculation. The composition of the mineral media used to feed the anode was that described by Parameswaran et al. (2009) modifying the electron donor concentration (SY and ethanol) as specified in Table 1; a 100 mM NaOH solution was used as the catholyte solution. The average initial pH of the media for each experiment was 7.3 while the cathode solution had an initial pH of 11.7. Negative and ethanol control experiments were also carried out: the ethanol control was performed by feeding 2400 mg COD/L ethanol to the MEC, while the negative control was performed by feeding only basal mineral media plus buffer. The duration of each experiment was not controlled but determined by current and gas production. Before each experiment, the eluent from the previous batch was completely removed and the reactors were washed out by recirculating a phosphate buffer solution (100 mM) for 30 min before adding new media. For each batch experiment the gas production was measured by the volume displaced using a 250 mL graduated cylinder inverted and placed in saline water at pH 2 to avoid CO_2 to dissolve, which can modify the real composition of the gas produced.

Table 1
Description of the experiments performed.

	Spent yeast (mg of COD/L)	Ethanol (mg of COD/L)
Ethanol control	0	2400
Exp. 1	750	0
Exp. 2	1500	0
Exp. 3	750	1200
Exp. 4	1500	1200
Negative control	0	0

Each in duplicate.

At the end of each experiment, samples were taken from the MEC in order to monitor TCOD, TSS, VSS, VFA, carbohydrates, proteins and pH. These parameters were quantified as described before. Moreover, biogas composition was monitored as follows: Gas composition (hydrogen, methane and carbon dioxide) was analyzed using a gas chromatographer (Model SRI 8619C, SRI Instruments, Scientific Repair Inc., Torrance, CA, USA) equipped with a Thermal Conductivity Detector (TCD) proceeding as explained by Hernández-Mendoza and Buitrón (2014).

2.6. Calculations

Based on the duration of each experiment batch, the rates of hydrogen production were estimated as $L/L_{reactor}$ per day, and the accumulated hydrogen mmols were calculated using the atmospheric conditions at which the experiments were performed (0.79 atm and 21 °C). Volumetric current density (A/m^3) was calculated using the effective volume of the anode and the surface current density (A/m^2) was estimated based on the approximated maximum surface of the electrode, considering 160,000 fibers with 7.2 μm diameter and 6.5 cm length, which gives a total area of 0.235 m^2. The same electrode geometry was used in all experiments thus the results for A/m^2 can be compared directly; however

Table 2
Chemical characterization of spent yeast from bench scale alcoholic fermentation.

	Immediately after fermentation (g/L)	2 weeks after fermentation (g/L)
Total COD	27.9	27.8
TSS	13.3	13.9
VSS	10.9	13.1
Total carbohydrates	8.9	6.9
Total proteins	3.7	9.5
$N\text{-}NH_3$	0.009	0.02
Ethanol	1.9	3.5
Acetate	0.6	1.1
Iso-butyrate	0.09	0.04
Butyrate	0.1	0.04
Iso-valerate	*	0.09
pH	4.17	3.38

* Below the detection limit.

these could not be compared to those obtained with other anode configurations. The organic matter removal efficiency (COD RE) was calculated as the fraction of the total COD that was removed from the fed substrate. The coulombic efficiency (CE) and coulombic recovery (CR) were estimated as expressed elsewhere (Mahmoud et al., 2014), calculating the accumulated coulombic production (C) for each experiment and available electrons from the fed substrate, using the following relationships:

$$8\ \text{g COD} = 1\text{e}^{-}\ \text{eq} \quad (1)$$

$$1\ \text{e}^{-}\ \text{eq} = 96485\ \text{C} \quad (2)$$

3. Results and discussion

3.1. Chemical characterization of SY

About 55.42 (±3) g/L of SY (wet weight) was recovered from the alcoholic fermentation to be used as substrate in the experiments. The chemical characteristics of the SY obtained are shown in Table 2. Total COD obtained (27.9 g/L) was lower than that reported in the literature (Bocher et al., 2008; Neira and Jeison, 2010). This is likely due to our use of malt extract during fermentation, which prevented the accumulation of more complex organic material from whole malt and hops (Huige, 2006). Furthermore, SY was assumed to be slowly biodegradable based on previous reports (Neira and Jeison, 2010), where the readily degradable fraction of SY COD is attributed to ethanol and other VFA's.

Changes in initial SY composition, as those reported in Table 2, could lead to different MEC treatment performance (Pant et al., 2010). Better performances, in terms of current density and hydrogen production, can be obtained when low TCOD concentrations rich in VFAs like acetate or simple sugars (Kadier et al., 2014) are fed to MECs. SY has high carbohydrate content (6.9 g/L) and low ammonium-nitrogen concentration (0.009 g of N-NH_3/L). That is very similar to the composition of brewery waste water that has also been tested in MFCs, and those characteristics could make it an ideal substrate for the process (Pant et al., 2010). In the same way, the presence of some volatile fatty acids like iso-butyrate and iso-valerate (0.04 and 0.09 g/L respectively) can indicate beer spoilage by wild yeast contamination, usually indicated by higher turbidity, excess gas, excess acidity and off-flavors (Varnam and Sutherland, 1999). These intermediary products are evidence of organic matter breakdown on anaerobic degradation processes (Rittmann and McCarty, 2001), indicating that degradation of SY could have already started. Ammonia content and acidic properties (pH 3.38) of the SY can influence the buffering capacity, so buffer addition can be advantageous for the process. A lower pH (<6) can inhibit ARB and thus limit current density in MECs (Torres et al., 2008). Complex molecules such as proteins and carbohydrates in high concentrations would require long treatments due to their slow biodegradability or alternatively necessitate the presence of a highly adapted microbial community (Rittmann and McCarty, 2001).

Table 2 also shows relevant variations detected in the SY two weeks before the experiments compared to the characteristics measured right before the experiments. While volatile suspended solids (VSS) and the total protein concentration increased with time (17% and 61%), the carbohydrate concentration decreased (28%) as well as ethanol was generated, which can be related to yeast activity affecting the stability of the substrate under storage conditions (Huige, 2006). Without an inactivation pretreatment, the SY contains damaged yeast (dead or with low viability) as well as live yeast. Under storage conditions, this leads to important changes in the composition of alcoholic fermentation products, like sugars and alcohols. Carbohydrates can decrease, while proteins increase due to glycogen metabolism of yeast cells (Huige, 2006). For this study, a SY that had been in storage for two weeks, with the characteristics listed in Table 2, was selected for the MEC treatment.

3.2. MEC overall performance

After the acclimation period and prior to the SY treatment experiments, an ethanol control, feeding only ethanol (2400 mg COD/L) as a substrate was evaluated. Additionally, a negative control was performed, feeding only basal mineral media without electron donors. Table 3 shows a summary of the results and performance parameters calculated for each experiment including ethanol control, while Fig. 2 displays the current density obtained for each experiment, including the negative control.

3.2.1. Current density and rate of hydrogen production

The highest maximum current density, 222.0 ± 31.3 A/m^3 (or 0.29 ± 0.04 A/m^2) was observed when feeding 1500 mg COD/L of SY and 1200 mg COD/L of ethanol (Exp. 3) as presented in Fig. 2 and Table 3. On the other hand, the lowest observed maximum value of current density, 87.8 ± 25.7 A/m^3 (or 0.12 ± 0.03 A/m^2), corresponds to the experiment with the lowest fed COD of 750 mg COD/L using SY as sole substrate (Exp. 1). The same trend was observed in terms of the rate of hydrogen production: 2.18 ± 0.66 L of $H_2/L_{reactor}$-d (22.2 ± 4.7 mmol of H_2/d) was registered when 1500 mg COD/L of SY and 1200 mg COD/L of ethanol (Exp. 4) were fed while only 0.64 ± 0.06 L of $H_2/L_{reactor}$-d (6.5 ± 0.4 mmol of H_2/d) was obtained in the first experiment feeding only 750 mg COD/L of SY (Exp. 1). A current density of 103.8 ± 9.3 A/m^3 was generated in the ethanol control experiment (ethanol 2400 mg COD/L) as well as a rate of 0.73 ± 0.12 L of $H_2/L_{reactor}$-d (7.4 ± 0.9 mmol of H_2/d) that is relatively higher than the treatment with only SY (Exp. 1). Likewise, a negative control was performed after running all the experiments to obtain the current density resulting from respiration of the ARB biofilm itself or from degradation of organic matter accumulated in the graphite brushes, reaching a maximum current density of 20.9 ± 2.8 A/m^3. These results are also shown in Fig. 2.

As observed in Fig. 2, the depletion of readily available substrate (as acetate) could be represented by the rate (shape of the curve) of current density production that occurs during the first hours of the treatment. Acetate was present at low concentration in SY (approx. 0.5 mM after dilution) and ARB have a half-maximum-rate concentration (Ks) for acetate of 0.04 mM (Esteve-Nuñez et al., 2005) which could probably outcompete acetoclastic methanogens that have a Ks ranging from 0.85 mM (Zinder, 1993) to 7 mM (Rittmann and McCarty, 2001) thus preventing methane production. The duration of the experiments was short (from 20 to 35 h when SY was fed) which could imply rapid utilization of the more available electron donors; this could also explain why methane was not dominantly produced, i.e. the low retention times limited its production (Hernández-Mendoza and Buitrón, 2014).

In the same way, time for adaptation could have an effect on the rate of substrate utilization (Rittmann and McCarty, 2001) for current production, as can be seen in Fig. 2, since higher rates were noticed on later experiments. It has been established that complexity of the substrate can affect the current density produced (Kadier et al., 2014; Pant et al., 2010; Torres et al., 2007). As an example from the literature, treating substrates like peptone and meat processing wastewater containing amino acids and proteins, had lower performance than using just bovine serum albumin (BSA) (Heilmann and Logan, 2006). Then, if more acclimation cycles with ethanol were performed, a more homogenous community of

Table 3
Performance parameters evaluated for the SY treatment. An average and standard deviation of the obtained results is shown.

	Ethanol control	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Coulombic efficiency (%)	78 ± 5	63 ± 3	63 ± 7	90 ± 2	71 ± 0.4
Coulombic recovery (%)	74 ± 6	46 ± 1	48 ± 4	81 ± 1	81 ± 1
COD removal efficiency (%)	96 ± 2	73 ± 8	76 ± 2	90 ± 1	87 ± 2
(max) A/m^3	103.8 ± 9.3	87.8 ± 25.7	131.4 ± 11.5	199.0 ± 8.0	222.0 ± 31.3
(max) A/m^2	0.14 ± 0.01	0.12 ± 0.03	0.17 ± 0.02	0.26 ± 0.01	0.29 ± 0.04
L of H_2/$L_{reactor}$-d	0.73 ± 0.12	0.64 ± 0.06	1.24 ± 0.46	1.73 ± 0.36	2.18 ± 0.66
mmol of H_2/d	7.4 ± 0.9	6.5 ± 0.4	12.6 ± 3.3	17.6 ± 2.6	22.2 ± 4.7
Initial pH	7.4	7.35	7.36	7.35	7.35
Final pH	6.88	7.22	7.00	6.83	6.80
Duration (h)	~115	~20	~20	~30	~35

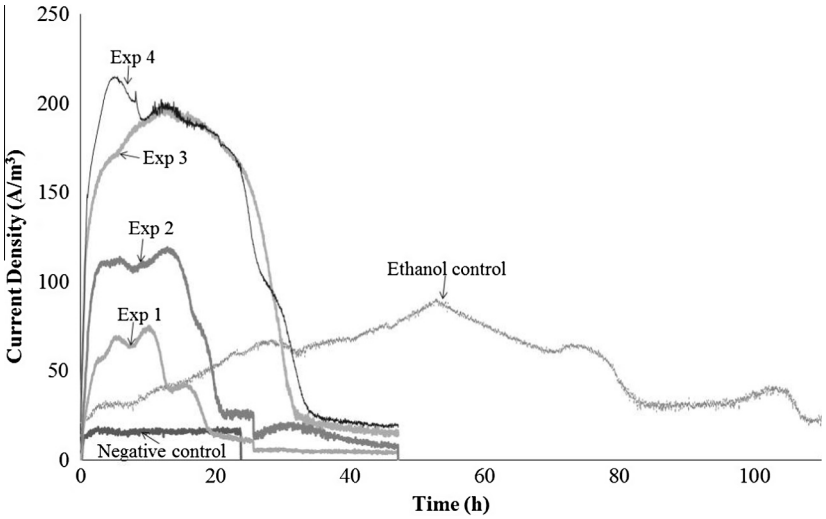

Fig. 2. Current density (A/m^3) produced in each experiment. The average of the duplicate experiments is shown here.

microorganisms would have been selected (Torres et al., 2007) limiting the utilization of more complex material like the SY.

Increasing the organic load (fed COD) seems to favor hydrogen production, obtaining higher rates when initial TCOD was higher, which implies that initial higher concentrations of simpler sole electron donor were available (Torres et al., 2007). Hydrogen production at the cathode was always dominant, and CO_2 was detected in a concentration lower than 1% only in those experiments where ethanol was fed (Exp. 3 and 4) and in the negative control.

3.2.2. Organic matter removal efficiency and electron recovery

Results of CE, CR and COD RE are shown in Table 3. In terms of electron recovery, the best results were 90 ± 2% of CE and 81 ± 1% of CR from the treatment using 750 mg COD/L of SY and 1200 mg COD/L of ethanol (Exp. 3). The second best electron recovery (78 ± 5% of CE and 74 ± 6% of CR) resulted when feeding 2400 mg COD/L of ethanol (ethanol control). On the other hand, SY as sole substrate had very similar results in both fed concentrations (63 ± 7% of CE and 48 ± 4% of CR for SY 1500 mg COD/L in Exp. 2 and 63 ± 3% of CE and 46 ± 1% of CR for SY 750 mg COD/L in Exp. 1) producing the lowest electron recoveries registered. The efficiency of the treatments to remove organic matter was represented by the calculated COD removal efficiency (COD RE%). A 90 ± 1% COD RE was obtained when feeding 750 mg COD/L of SY and 1200 mg COD/L ethanol (Exp. 3); a relatively lower performance was obtained when a higher organic load was fed (i.e. 87 ± 2% when feeding 1500 mg COD/L of SY plus 1200 mg COD/L ethanol in Exp. 4). However lower efficiencies were obtained when feeding only SY (73 ± 8% for 750 mg COD/L of SY in Exp. 1 and 76 ± 2% for 1500 mg COD/L in Exp. 2). The ethanol control gave the best results, reaching 96 ± 2% of COD RE.

Compared to the ethanol control performed here, higher CEs (86%) have been previously obtained by Parameswaran et al. (2009) when feeding only ethanol, which could be related to acclimation, to the MEC set up conditions in this study, and high internal resistance of the MEC, which could lower process performance (Hu, 2008). From COD RE, CE and CR results (Table 3) it could be concluded that the treatment process was predominantly fermentative. A high percentage of the fed COD was utilized or retained in the reactor but it could not be completely used to produce current. Since the ethanol concentration at the end of each experiment (including ethanol control) was below the detection limit, it can be assumed that ethanol COD (or electrons equivalent) was consumed completely, which could also explain the accumulation of acetate at the end of each batch due to fermentation (Torres et al., 2007). The length of time of each batch was not enough to achieve complete utilization of the generated VFAs that were also detected at the end of the treatments, thus the biomass concentration of ARB present in the system could be low or be inhibited by organic load. Since ARB prefer utilizing simple compounds as acetate or hydrogen, the initially utilized fraction of available electrons might correspond mainly to the acetate present in the SY (Table 2 and Table 4) and acetate produced from fermentation.

3.2.3. Statistical analysis

An analysis of variance of the results was performed with the intention of determining the influence of SY concentration and ethanol addition on the treatment performance. The response variables selected for the statistical analysis were the CE, COD

Table 4
Mass and electron balances calculation for the experiments and control.

	Exp. 1			Exp. 2			Exp. 3			Exp. 4			Ethanol control		
	COD (mg)	%	meq e-	COD (mg)	%	meq e-	COD (mg)	%	meq e-	COD (mg)	%	meq e-	COD (mg)	%	meq e-
Initial COD	233	100	29	465	100	58	605	100	76	837	100	105	744	100	93
(Fed acetate)			1.2			2.5			1.2			2.5			0
Final TCOD	62	27	8	113	24	14	59	10	7	110	13	14	31	4	4
Current	108	46	13	221	48	28	491	81	61	514	61	64	554	74	69
Other COD sinks	63	27	8	131	28	16	55	9	8	213	25	27	159	22	20

RE. Ethanol addition had a significant effect on the performance of the process, specifically on COD RE (p value = 0.011 < 0.05) and on CE (p value = 0.012 < 0.05); however it cannot be concluded statistically whether SY concentration had an effect on these parameters (p value > 0.05). Consequently the optimal condition for SY treatment is ethanol addition and in this study, having a higher concentration of ethanol than SY was more favorable, as observed in Exp. 3.

From this analysis, it can be inferred that the microbial community was better adapted to using ethanol than SY as a sole substrate, using it either to produce acetate or directly as an electron donor. To evaluate the effect of SY concentration, higher loads should be tested to find the overcharge point of the process. Compared to simple substrates, such as acetate or ethanol, slow hydrolysis steps are required to produce usable electron donors from complex substrates (Kadier et al., 2014) such as SY. Therefore, better results were obtained due to electron availability when ethanol was used, as the SY has to be transformed to simpler compounds, and this degradation process includes more reactions and more possible electron sinks.

3.3. Mass and electron balances

Production of current in MECs is linked to the ability of bacteria to oxidize a substrate and transfer the resulting electrons to the anode electrode. A mass and electron balance for the experiments is presented in Table 4, where the estimated distribution of the fed electrons is displayed. The percentage of electrons lost due to unknown sinks was about the same (27 ± 1%) when feeding SY as sole substrate (750 mg COD/L in Exp. 1 and 1500 mg COD/L in Exp. 2 respectively) implying that electron recovery was independent from SY concentration. On the other hand, 25% of the electrons fed were lost when feeding the higher concentration of SY (1500 mg COD/L) in combination with ethanol (1200 mg COD/L) (Exp. 4) while 9% were lost when feeding SY (750 mg COD/L) and ethanol (1200 mg COD/L) (in Exp. 3); this could imply that ethanol addition can be an advantage to gain electrons from SY degradation when the concentration of ethanol is higher than that of SY.

The unknown sinks have an important role in the understanding of the treatment results. In the absence of exogenous electron acceptors (e.g., oxygen, nitrate, and sulfate), H_2, CH_4, biomass, and soluble microbial products (SMPs) are the major electron sinks that can divert electrons from electric current (Parameswaran et al., 2009). Intermediary products that are charged negatively like acetate or butyrate have been noticed to migrate to the cathode chamber when using AEM in MFCs (Pandit et al., 2012) and in MECs, which could also be considered a minor electron sink (Sleutels et al., 2009) in the experiments performed.

A low production of biogas (presumably methane) was noticed at the anode (less than 5 mL per batch, but not analyzed because it was below the equipment's minimum volume requirement of 10 mL). Based on the electron balance, methanogenesis does not represent a significant electron sink, since 5 mL of methane would represent 2 meq of electrons, corresponding to 3.5% of the total of fed electrons lost to unknown electron sinks in the experiment with the highest lost (28% of the fed mg of COD when feeding 1500 mg COD/L of SY in Exp. 2).

Since high surface anodes were used, biomass growth could be the most important electron sink, and given the complexity of the substrate, enough area was also available for the growth of a very diverse community (Logan et al., 2007) that could be mostly fermentative (Pant et al., 2010; Torres et al., 2007). A high surface was used to improve mass transfer, but it may have been responsible for the accumulation of organic matter in the system that can be related to high COD RE but relatively low CE and CR. For future work, clogging of the brush electrode should be tested as well as the effects of providing excessive surface on MEC systems performance.

3.4. Evidences of SY degradation

3.4.1. Solids, proteins and carbohydrates

As shown in Fig. 3, the TSS and VSS concentration decreased in all the experiments being lower when higher organic loads were fed. When SY was fed as sole substrate, being 750 mg COD/L in Exp. 1 and 1500 mg COD/L in Exp. 2, the TSS decreased by 77% and 87% respectively. When 750 mg COD/L of SY was fed in combination with 1200 mg COD/L of ethanol (Exp. 3) the TSS decreased by 79% and by 92% when the higher load of COD and SY was fed (1500 mg COD/L and 1200 mg COD/L of ethanol in Exp. 4). The VSS concentration decreased by 84 and 94% when SY was fed as a sole substrate (750 mg COD/L in Exp. 1 and 1500 mg COD/L in Exp. 2 respectively). Likewise, the VSS decreased by 84% and 93% when SY was fed in combination with 1200 mg COD/L of ethanol (750 mg COD/L in Exp. 3 and 1500 mg COD/L in Exp. 4 respectively). Carbohydrate degradation seemed to be independent from the organic loading rate: 89% and 92% of carbohydrates were

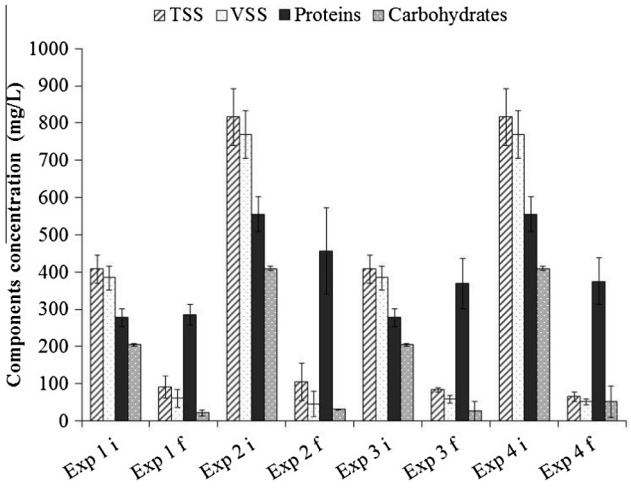

Fig. 3. Concentration of the monitored SY components. This figure presents the results for TSS, VSS, proteins and carbohydrates concentration measured initially (i) and at the end (f) of each experiment, and are expressed in mg/L. The average of the results obtained and the standard deviation are shown.

consumed when 750 mg COD/L (Exp. 1) and 1500 mg COD/L (Exp. 2) of SY were fed, respectively, and 87% of the fed carbohydrates were consumed in the experiments where both SY and ethanol were used (Exp. 3 and 4). An important accumulation in proteins concentration can be observed in Fig. 3, from Exp. 3, when 750 mg COD/L of SY and 1200 mg COD/L of ethanol was utilized (33% higher than fed concentration). However an increase in soluble proteins could be related to yeast activity, the release of proteolytic enzymes (Lee, 1996) and possible autolysis (Vosti and Joslyn, 1952) which corresponds to previous studied changes in SY composition under storage conditions (Huige, 2006) and the experiments performed in this study.

3.4.2. Fermentation products

In anaerobic degradation of food processing wastes, the major fermentation substrates are amino-acids that are released from protein hydrolysis. These amino-acids can be oxidized to acetate, butyrate, iso-valerate, iso-butyrate and valerate and other VFAs through Stickland fermentation (Batstone et al., 2003). Since SY has a high protein concentration this pathway of degradation could have been followed. As shown in Fig. 4, acetate was only totally consumed when 1500 mg COD/L of SY were fed in Exp. 2. An accumulation of acetate was noticed in both experiments where SY combined with ethanol was fed (Exp. 3 and 4) indicating that the ARB could be inhibited by the higher organic load and greater fermentation that lowered pH (Table 3). When feeding only ethanol (2400 mg COD/L) the highest accumulation of acetate was noticed confirming fermentation and also possible inhibition. Butyrate concentration was significant in the analyzed eluent only when ethanol was fed as a sole substrate (ethanol control) and in Exp. 1, when SY was first fed as a sole substrate. Thus products from the previous experiment could have remained in spite of having washed the reactors. Higher concentrations of iso-butyrate and iso-valerate remained after the treatment of 750 mg COD/L of SY and 1200 mg COD/L of ethanol in Exp. 3, but these were mostly consumed in the following experiments. Moreover, only this experiment exhibited an increase in soluble proteins (33%) which could be an evidence of proteolysis and amino-acid fermentation. pH declined slightly at the end of the treatments, decreasing 7.5% when accumulation of VFA was also noticed (Fig. 4, ethanol control, Exp. 3 and 4) and with increased organic load (7% decrement was noticed in the ethanol control). However, this can also be related to protons accumulation at the anode, since higher pH changes were observed when higher hydrogen production was also noticed (Table 3).

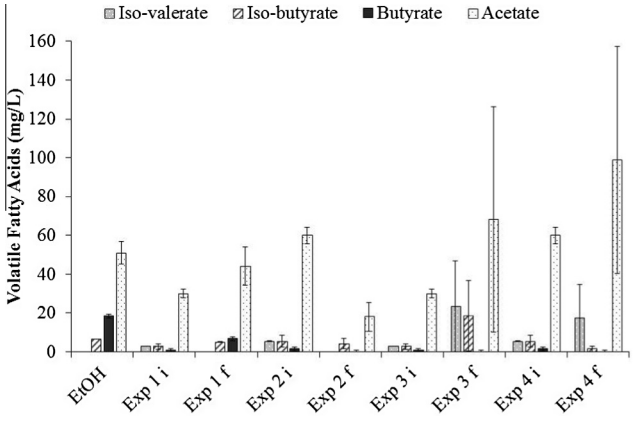

Fig. 4. Concentration of fermentation products (VFA) from SY treatment. This figure presents the results of VFA concentrations measured initially (i) and at the end (f) of each experiment and are expressed in mg/L. The average of the obtained results and the standard deviation are shown. "EtOH" labeled bars correspond to the measured VFAs at the end of the ethanol control experiment.

3.4.3. Effect of the MEC experimental conditions

The proposed MEC treatment of yeast biomass requires cell wall disruption to make cell constituents available to the microbial community at the anode. On regular yeast extract preparations, yeast lysis can be accomplished by autolysis (Kim et al., 1999; Reed and Nagodawithana, 1991; Shotipruk et al., 2005), which depends on temperature and pH (Vosti and Joslyn, 1952) as well as plasmolysis driven by high concentrations (0.5–0.95 g solvent/g packed yeast) of some solvents miscible in water, such as methanol, ethanol, iso-propanol, t-butanol (Fenton, 1982). Likewise, autolysis indicators have been shown to be optimal near neutral pH due to enhanced peptidase activity (Vosti and Joslyn, 1952). Thus, ethanol addition and neutral pH can be advantageous for SY breakdown.

Additionally, the effect of an MEC electrostatic field on the degradation of yeast cells should be explored. A previous study (Vu et al., 2011) showed that at an electrode potential of −0.3 V vs. Ag/AgCl, yeast cells that were previously adhered to a carbon paste electrode, detached, probably due to charge repulsion, since the yeast cell wall is negatively charged. The impact that this could have on the results obtained in the present study is not clear and should be evaluated in future work.

Taken together, the results obtained in this work offer an insight for future real utilization of SY from breweries in MEC to generate energy, and particularly hydrogen, to obtain value from this waste. This was an exploratory study on SY treatment using MECs. Improvements in reactor design and operation suggest that better performance can be achieved; however, more technological advances are required on the way towards large-scale applications.

4. Conclusions

A better performance was obtained when treating a mixture of SY and ethanol than when treating SY alone: 90 ± 2% CE, 81 ± 1% CR and 90 ± 1% COD RE were achieved when feeding 750 mg COD/L SY and 1200 mg COD/L ethanol, compared to 63 ± 3% CE and 46 ± 1% CR and 73 ± 8% COD RE when feeding 750 mg COD/L SY. However, the highest current densities and hydrogen productions were obtained with higher organic loads, although with lower CE, CR and COD RE, thus MEC treatment of SY can be improved with ethanol addition (p value = 0.011), by autolysis, and acclimation of the bacterial population.

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